Effects of Low Frequency Magnetic Fields on DNA Synthesis

doi: 10.1109/20.539508

EFFECTS OF LOW FREQUENCY MAGNETIC FIELDS ON DNA SYNTHESIS

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Abstract - **50/60Hz magnetic fields are of special concern in biomagnetics because living organisms are constantly exposed to these fields. We report the influences of a very low frequency alternating-current magnetic stimulus as high as 1.0T on the DNA synthesis processes *in vitro*. In this study, we examined both the activity of enzyme and the DNA synthesis error rate. Enzyme activity and synthesis error rate are measured by the speed of DNA synthesis and the number of mistaken DNA pairs respectively. In these experiments we used artificially synthesized DNA and a radioisotope technology. By comparing the *exposed* and *control* cases, we cannot recognize any differences for either the enzyme activity or error rate.**

I. INTRODUCTION

It has been reported recently that low frequency alternating magnetic fields may be harmful to live organisms [1],[2]. However, it is not clear whether or not these effects are in fact true. In order to provide further supporting evidence, it is necessary to identify the influence of magnetic fields on biochemical reactions. In our group we have been studying the influence of magnetic fields on a kind of nematode (*C.elegance*) [3]. In this paper we investigate the influence of magnetic fields on fundamental biochemical reactions *in vitro*. In order to understand the influence of magnetic fields on live organisms, it is important to investigate the influence of the magnetic field on each biochemical reaction. The experiments *in vitro* have the advantage of being reproducible and being able to limit the reaction which we want to observe. Therefore it becomes easier to provide a quantitative discussion, and to ascertain whether or not the magnetic field has any influence. We carried out this experiment with a 60Hz alternating magnetic field stimuli as high as 1.0T. The enzyme which catalyzes the DNA synthesis reaction is referred to as DNA polymerase. In this experiment we used two types of DNA polymerase,"klenow" and "exo(-) klenow", which are made from Escherichia coli's DNA polymerase. Also the speed of DNA synthesis and the synthesis error rate dependence on magnetic fields was carried out.

Manuscript received March 1, 1996.
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II. EXPERIMENTAL PRINCIPLE

DNA has double-helix structure, and each strand is a complement of the other. Therefore double strand DNA can be reproduced from single strand DNA. In our experiments we have synthesized DNA.

As shown in Fig.1, we prepared single strand template DNA made from 300 to 400 base deoxyadenylic acid. As the template DNA is made from only deoxyadenylic acid, deoxythymidine 5'-triphosphate (dTTP) is incorporated separately. By measuring the amount of dTTP within the DNA, we can know the speed of DNA synthesis. Labeling the dTTP with ^{3}H (tritium), a radioisotope tracer, allows us to measure the amount of dTTP used to synthesize the DNA by determining the integrated radiation.

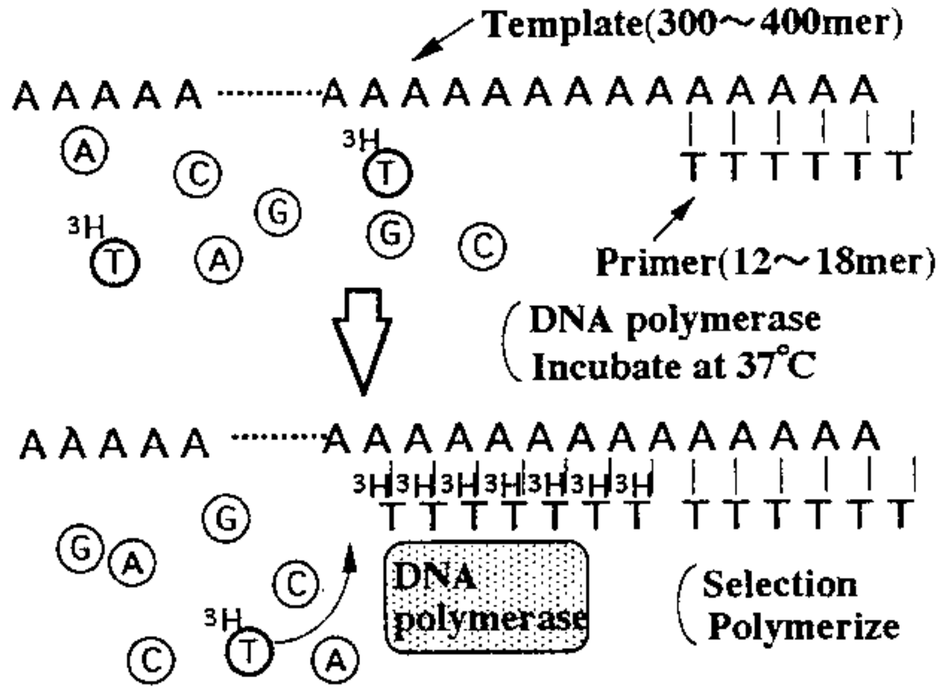

Fig.1 Principle of the speed of DNA synthesis measurement.

DNA polymerase has two functions to perform; one is to select the deoxyribonucleoside 5'-triphosphate which is complementary to DNA; the second is to polymerize it into DNA. We used two types of DNA polymerase in a series of experiments. Klenow has two activities. One activity is to select and polymerize deoxyribonucleoside 5'-triphosphate into DNA. The second activity is to cut off deoxyribonucleotide from DNA if an incorrect deoxyribonucleotide is polymerized. The latter activity is called a 3'-5' exonuclease activity. Exo(-) klenow does not generate the latter form of reaction. It is thought that exo(-) klenow generates additional

incorrect nucleotides compared to klenow.

The electromagnet which was used in this experiment is composed of two E-type cores with their poles placed face to face. The experimental space consists of a 13mm gap in the center leg. By supplying a 60Hz, 190A current, we can produce an AC magnetic field with a peak flux density of 1.0T. It has been confirmed that the magnetic field within the experimental space is uniform to within 2%. The conductivity of the buffer was measured to be 0.12 S/m, then the maximum induced current density in the experiment tube with a 7 mm diameter was estimated to be 0.24A/m2 at a 1.0T, 60 Hz magnetic field. We set an incubator in this gap, and kept the incubator temperature at 37°C by circulating water through the incubator chamber.

III. The Influence on The Speed of DNA Synthesis

We adjusted the sample as shown in Table I. In this sample four types of deoxyribonucleoside 5'-triphosphate are adjusted at the same concentration. Thus in this experiment, DNA polymerase is necessary to select the complementary deoxyribo-nucleoside 5'-triphosphate from four types.

Fig.2 shows the experimental procedure. First we mix all of the ingredients except the DNA polymerase, and incubate the mixture at 37°C for 5 minutes in advance. After the DNA polymerase is added, we expose the sample to the 1.0T AC magnetic field. At the end of an incubation interval we stop the reaction by mixing the sample with 10% TCA (trichloroacetic acid) so as to inactivate DNA polymerase. Finally we filtrate the sample to wash out deoxyribo-nucleoside 5'-triphosphate which is not a part of DNA, and then determine the total amount of radiation received from DNA using a liquid scintillation counter. From the radiation counts we can determine the length of DNA which was synthesized.

Table I Concentration of sample (1).

Ingredient	Final concentration
Template-Primer(Liq.)	100 pM
KPO_4	67 mM
$MgCl_2$	6.7 mM
2-mercaptoethanol	1.0 mM
dATP	40 μM
dCTP	40 μM
dGTP	40 μM
^{3}H-dTTP+dTTP	40 μM
DNA polymerase	0.15 U

(M=mol/ℓ)

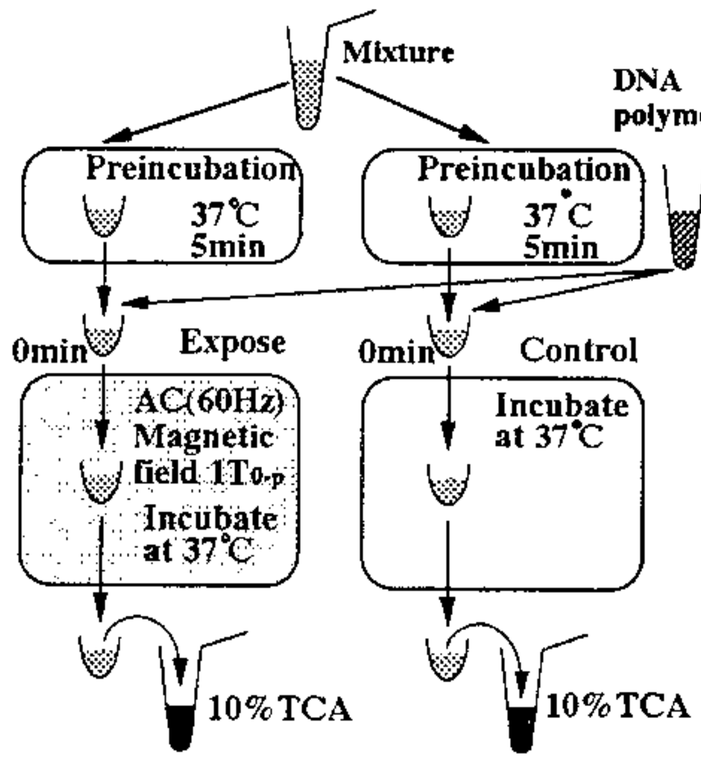

Fig.2 Experimental procedure.

Fig.3 shows the results when klenow is used as DNA polymerase. This figure shows six mean times, and the error bars representing the standard division. There appears to be no difference between the *expose* sample and the *control* sample. The results using exo(-) klenow are shown in Fig.4. For this case no difference was observed. From these results we conclude that a 60Hz 1.0T magnetic field has no effect on the DNA synthesis speed *in vitro*. In particular, the function to polymerize deoxyribonucleoside 5'-triphosphate is not influenced by magnetic fields.

IV. Error Rate Dependence on Magnetic Fields

We investigated the magnetic fields influence on the DNA synthesis error rate. The error refers to the generation of an uncomplimentary deoxyribonucleoside 5'-triphosphate which are incorporated within the template DNA.

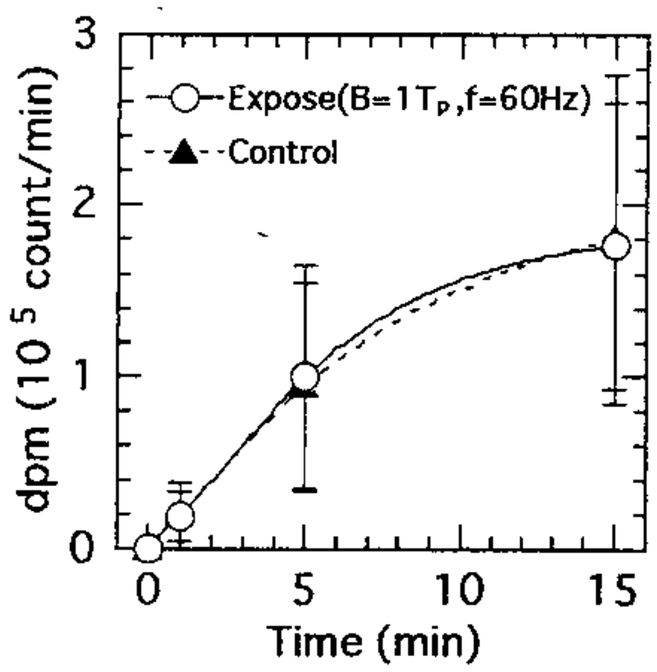

Fig.3 Influences on the speed of DNA synthesis when klenow is used.

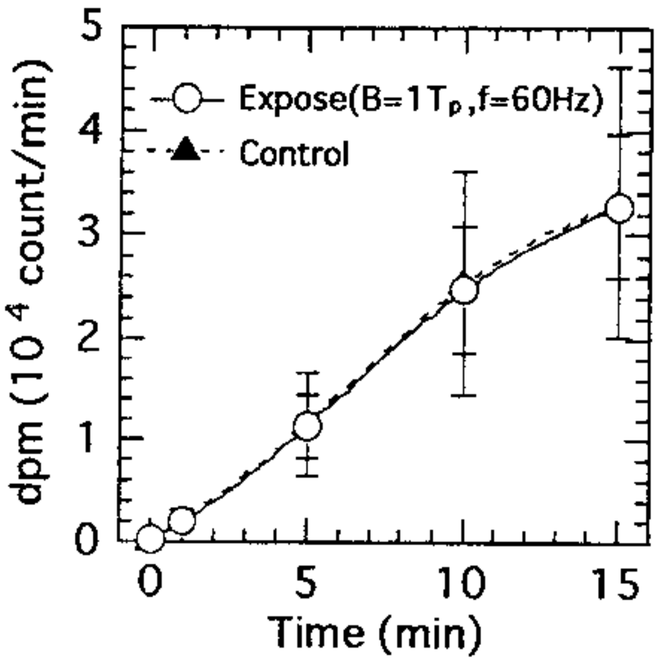

Fig.4 Influences on the speed of DNA synthesis when exo(-) klenow is used.

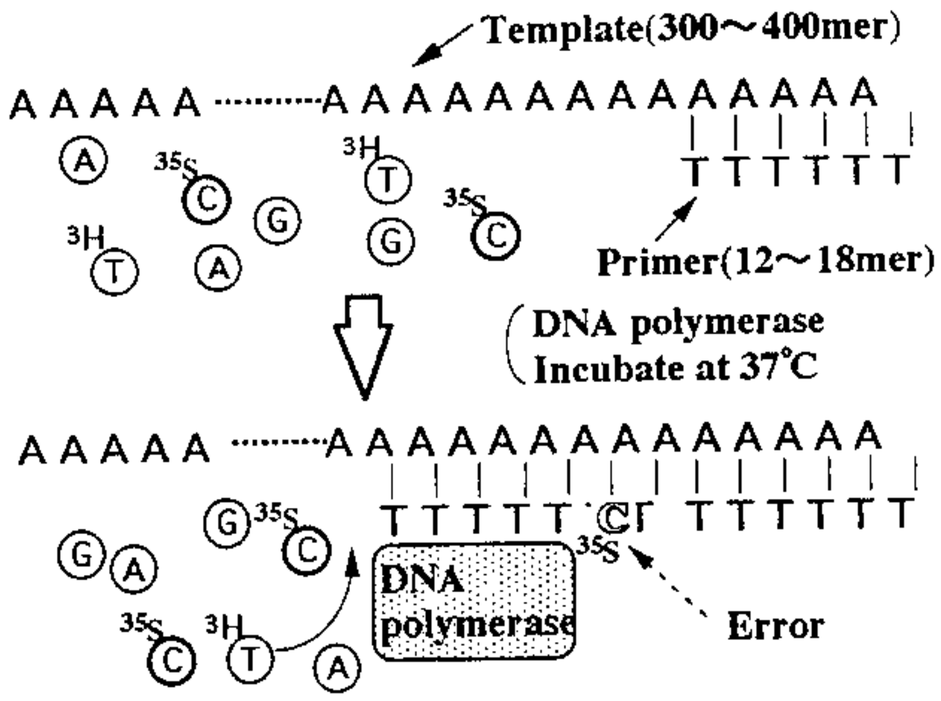

Fig.5 Principle of error rate measurement.

Table II Concentration of sample (2).

Ingredient	Final concentration
Template-Primer(Liq.)	100 pM
KPO_4	67 mM
$MgCl_2$	6.7 mM
2-mercaptoethanol	1.0 mM
dATP	40 μM
[α-^{35}S] dCTP	40 μM
dGTP	40 μM
^{3}H-dTTP+dTTP	40 μM
DNA polymerase	0.15 U

(M=mol / ℓ)

The method of performing this experiment is similar to the method for obtaining the speed of DNA synthesis. In this experiment as shown in Fig.5, we used deoxycytidine 5'-triphosphate (dCTP) which is labeled with ^{35}S. Incorrectly incorporated dCTP can be determined by measuring the emitted radiation. Table II shows the concentrations of the ingredients.

Fig.6 shows the results when klenow was used. The result represents the mean of three experiments. From this result, it is clear that the error rate is not influenced by magnetic fields. The results by using exo(-) klenow are shown in Fig.7. In this case the error rate is larger compared to the case when klenow was used. This is thought to occur because exo(-) klenow has no 3'-5' exonuclease activity. Therefore more errors are generated compared to the case when klenow was used. Thus both results have high error rates at 0 minute and after 1 minute. We consider this result to be caused by dCTP which is attached to the filter. In other words, the reaction time is short, so incorporated materials are small and thus the radiation is weak. Therefore the radiation from the remaining dCTP which has no connection with the DNA product affects the result.

From these results, we can conclude that low frequency alternating current magnetic fields do not generate errors during DNA synthesis. In particular, it becomes clear that the function of selection and 3'-5' exonuclease activity are not effected by magnetic fields in this experiment.

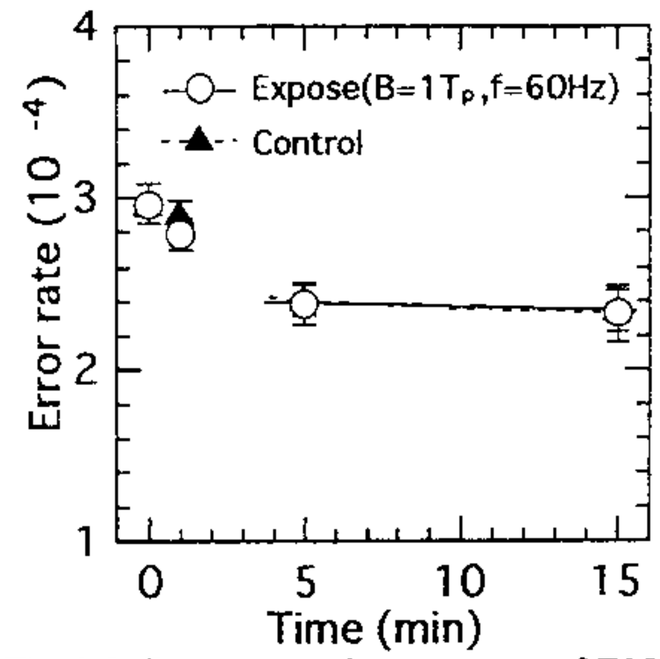

Fig.6 Influences on the error rate of DNA synthesis when klenow is used.

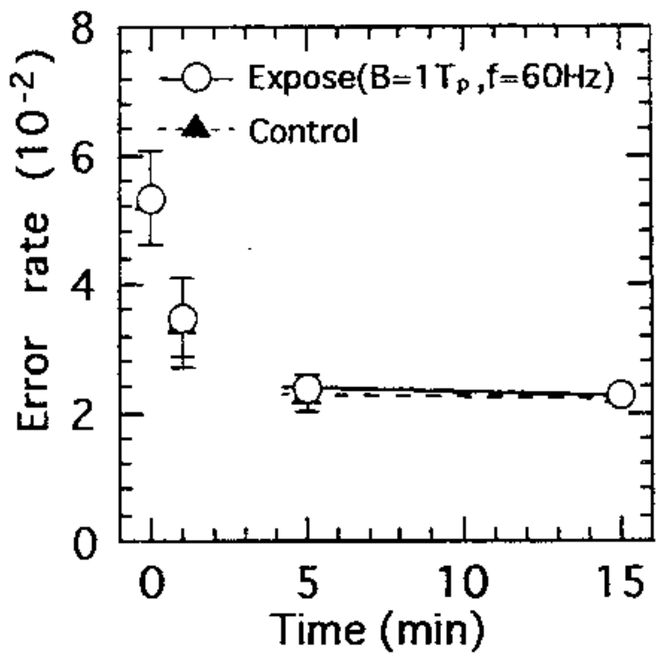

Fig.7 Influences on the error rate of DNA synthesis when exo(-) klenow is used.

V. Conclusion

We examined the influence of a 60Hz 1.0T alternating current magnetic field on DNA synthesis *in vitro*. Observations indicate that the speed of DNA synthesis and the error rate for both DNA polymerase are not influenced by magnetic fields. From these results, we conclude that DNA polymerase's two functions to select the complementary deoxyribonucleoside 5'-triphosphate and to polymerize it into DNA are not effected by low frequency magnetic fields with peak intensities less than 1.0T.

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